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(54) Title: NUCLEOSIDES WITH ANTI-HEPATITIS B VIRUS ACTIVITY (57) Abstract A method for the treatment of a host, and in particular, a human, infected with HBV is provided that includes administering an HBV-treatment amount of the stabilized nucleotide of a nucleoside which exhibits anti-hepatitis B activity.		

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NUCLEOSIDES WITH ANTI-HEPATITIS B VIRUS ACTIVITY

Background of the Invention

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This invention is in the area of methods for the treatment of hepatitis B virus (also referred to as "HBV") that includes administering an effective amount of one or more of the active compounds disclosed herein, or a pharmaceutically acceptable derivative or prodrug of one of these
10 compounds.

HBV is second only to tobacco as a cause of human cancer. The mechanism by which HBV induces cancer is unknown, although it is postulated that it may directly trigger tumor development, or indirectly trigger tumor development through chronic inflammation, cirrhosis, and
15 cell regeneration associated with the infection.

Hepatitis B virus has reached epidemic levels worldwide. After a two to six month incubation period in which the host is unaware of the infection, HBV infection can lead to acute hepatitis and liver damage, that causes abdominal pain, jaundice, and elevated blood levels of certain
20 enzymes. HBV can cause fulminant hepatitis, a rapidly progressive, often fatal form of the disease in which massive sections of the liver are destroyed. Patients typically recover from acute viral hepatitis. In some patients, however, high levels of viral antigen persist in the blood for an extended, or indefinite, period, causing a chronic infection. Chronic
25 infections can lead to chronic persistent hepatitis. Patients infected with chronic persistent HBV are most common in developing countries. By mid-1991, there were approximately 225 million chronic carriers of HBV in Asia alone, and worldwide, almost 300 million carriers. Chronic persistent hepatitis can cause fatigue, cirrhosis of the liver, and

hepatocellular carcinoma, a primary liver cancer. In western industrialized countries, high risk groups for HBV infection include those in contact with HBV carriers or their blood samples. The epidemiology of HBV is in fact very similar to that of acquired immunodeficiency syndrome, which
5 accounts for why HBV infection is common among patients with AIDS or HIV-associated infections. However, HBV is more contagious than HIV.

Daily treatments with α -interferon, a genetically engineered protein, has shown promise. A human serum-derived vaccine has also been developed to immunize patients against HBV. Vaccines have been
10 produced through genetic engineering. While the vaccine has been found effective, production of the vaccine is troublesome because the supply of human serum from chronic carriers is limited, and the purification procedure is long and expensive. Further, each batch of vaccine prepared from different serum must be tested in chimpanzees to ensure safety. In
15 addition, the vaccine does not help the patients already infected with the virus.

European Patent Application No. 92304530.6 discloses that a group of 1,2-oxathiolane nucleosides are useful in the treatment of hepatitis B infections. It has been reported that the 2-hydroxymethyl-5-(cytosin-1-yl)-
20 1,3-oxathiolane has anti-hepatitis B activity. Doong, et al., Proc. of Natl. Acad. Sci. USA, 88, 8495-8499 (1991); Chang, et al., J. of Biological Chem., Vol 267(20), 13938-13942. The anti-hepatitis B activity of the (-) and (+)-enantiomers of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane has been published by Furman, et al., in Antimicrobial Agents and Chemotherapy, Dec. 1992, pages 2686-2692.
25

PCT/US92/03144 (International Publication No. WO 92/18517) filed by Yale University discloses a number of β -L-nucleosides for the treatment of both HBV and HIV. Other drugs explored for the treatment of HBV include adenosine arabinoside, thymosin, acyclovir, phosphonoformate,

zidovudine, (+)-cyanidanol, quinacrine, and 2'-fluoroarabin syl-5-iodouracil.

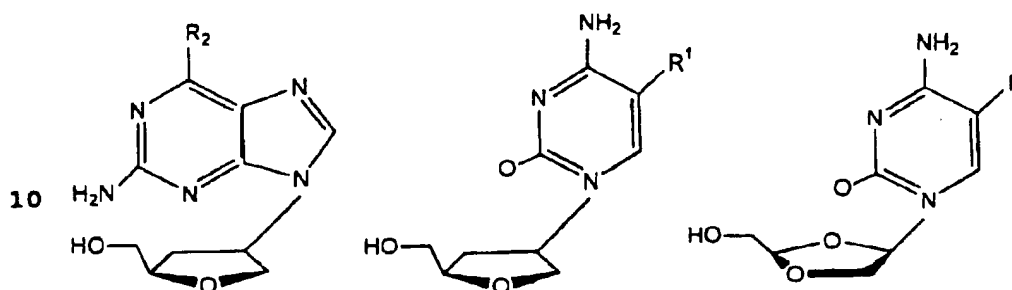
An essential step in the mode of action of purine and pyrimidine nucleosides against viral diseases, and in particular, HBV and HIV, is their metabolic activation by cellular and viral kinases, to yield the mono-, di-, and triphosphate derivatives. The biologically active species of many nucleosides is the triphosphate form, which inhibits DNA polymerase or reverse transcriptase, or causes chain termination. The nucleoside derivatives that have been developed for the treatment of HBV and HIV to date have been presented for administration to the host in unphosphorylated form, notwithstanding the fact that the nucleoside must be phosphorylated in the cell prior to exhibiting its antiviral effect, because the triphosphate form has typically either been dephosphorylated prior to reaching the cell or is poorly absorbed by the cell. Nucleotides in general cross cell membranes very inefficiently and are generally not very potent *in vitro*. Attempts at modifying nucleotides to increase the absorption and potency of nucleotides have been described by R. Jones and N. Bischofberger, *Antiviral Research*, 27 (1995) 1-17, the contents of which are incorporated herein by reference.

In light of the fact that hepatitis B virus has reached epidemic levels worldwide, and has severe and often tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat humans infected with the virus that have low toxicity to the host.

Therefore, it is another object of the present invention to provide a method and composition for the treatment of human patients or other hosts infected with HBV.

Summary of the Invention

A method for the treatment of a host, and in particular, a human, infected with HBV is provided that includes administering an HBV-
 5 treatment amount of a nucleoside of the formula:



wherein:

R^1 is hydrogen, fluoro, bromo, chloro, iodo, methyl or ethyl; and R^2
 15 is OH, Cl, NH_2 , or H; or a pharmaceutically acceptable salt of the compound, optionally in a pharmaceutically acceptable carrier or diluent.

In an alternative embodiment, the β -L-enantiomer of a compound of the formula:

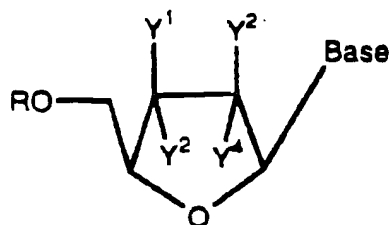
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wherein R^5 is adenine, xanthine, hypoxanthine, or other purine, including an alkylated or halogenated purine is administered to a host in an HBV-treatment amount as described more fully herein.

In another alternative embodiment, the nucleoside is of the formula:



wherein B is a purine or pyrimidine base;

Y^1 , Y^2 , Y^3 , and Y^4 are independently H, OH, N_3 , NR^1R^2 , NO_2 ,
 10 NOR^3 , -O-alkyl, -O-aryl, halo (including F, Cl, Br, or I), -CN, -
 $C(O)NH_2$, SH, -S-alkyl, or -S-aryl, and wherein typically three of Y^1 , Y^2 ,
 Y^3 , and Y^4 are either H or OH. The -OH substituent, when present, is
 typically a Y^1 or Y^3 group. As illustrated in the structure, Y^2 and Y^4 are in
 the arabino (erythro) configuration, and Y^1 and Y^3 are in the threo (ribose)
 15 configuration. R is H, monophosphate, diphosphate, triphosphate, alkyl,
 acyl or a phosphate derivative, as described in more detail below. R^1 , R^2 ,
 and R^3 are independently alkyl (and in particular lower alkyl), aryl,
 aralkyl, alkaryl, acyl, or hydrogen.

In a preferred embodiment, the nucleoside is provided as the indicated
 20 enantiomer and substantially in the absence of its corresponding enantiomer
 (i.e., in enantiomerically enriched form).

In another embodiment, the invention includes a method for the
 treatment of humans infected with HBV that includes administering an
 HBV treatment amount of a prodrug of the specifically disclosed
 25 nucleosides. A prodrug, as used herein, refers to a pharmaceutically
 acceptable derivative of the specifically disclosed nucleoside, that is
 converted into the nucleoside on administration *in vivo*, or that has activity
 in itself. Nonlimiting examples are the 5' and N^4 -pyrimidine or N^6 -purine
 acylated or alkylated derivatives of the active compound.

In a preferred embodiment of the invention, the nucleoside is provided as the monophosphate, diphosphate or triphosphate in a formulation that protects the compound from dephosphorylation. Formulations include liposomes, lipospheres, microspheres or nanospheres (of which the latter
5 three can be targeted to infected cells). In an alternative preferred embodiment, the nucleoside is provided as a monophosphate, diphosphate or triphosphate derivative (i.e., a nucleotide prodrug), for example an ester, that stabilizes the phosphate in vivo. In an alternative embodiment of this invention, a stabilized phosphate derivative, as described further
10 below, of FTC, BCH-189, or 3TC is provided for the treatment of hepatitis.

The disclosed nucleosides, or their pharmaceutically acceptable prodrugs or salts or pharmaceutically acceptable formulations containing these compounds are useful in the prevention and treatment of HBV
15 infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in
20 individuals who are anti-HBV antibody or HBV-antigen positive or who have been exposed to HBV.

In one embodiment of the invention, one or more of the active compounds is administered in alternation or combination with one or more other anti-HBV agents, to provide effective anti-HBV treatment. Examples
25 of anti-HBV agents that can be used in alternation or combination therapy include but are not limited to the 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC", see WO 92/14743), its physiologically acceptable derivative, or physiologically acceptable salt; the 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (including the racemic BCH-189 form, or

3TC (BCH-189 enriched with the (-)-enantiomer) its physiologically acceptable derivative, or physiologically acceptable salt; 2'-fluoro-5-ethyl-arabinosyluracil (FEAU); carbovir, or interferon.

Any method of alternation can be used that provides treatment to the patient. Nonlimiting examples of alternation patterns include 1-6 weeks of administration of an effective amount of one agent followed by 1-6 weeks of administration of an effective amount of a second anti-HBV agent. The alternation schedule can include periods of no treatment. Combination therapy generally includes the simultaneous administration of an effective ratio of dosages of two or more anti-HBV agents.

In light of the fact that HBV is often found in patients who are also anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV, the active anti-HBV compounds disclosed herein or their derivatives or prodrugs can be administered in the appropriate circumstance in combination or alternation with anti-HIV medications, including but not limited to 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (FTC), or 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (racemic BCH-189 or BCH-189 enriched with the (-)-enantiomer, 3TC). Non-nucleoside RT-inhibitors such as the Tibo class of compounds, nevirapine, or pyrimidinone can also be administered in combination with the claimed compounds.

The active anti-HBV agents can also be administered in combination with antibiotics, other antiviral compounds, antifungal agents, or other pharmaceutical agents administered for the treatment of secondary infections.

In one embodiment, the nucleoside is provided as a phosphate derivative that is stabilized to decrease or eliminate dephosphorylation prior

to uptake into the infected cell. A number of stabilized phosphate derivative groups in the 5'-position of the nucleoside are known and have been published in the literature. In one embodiment, the nucleoside is administered as a SATE derivative, as disclosed in more detail below. Any
5 alternative stabilized phosphate derivative can be placed in the 5'-position of the nucleoside that does not materially adversely affect the activity of the compound.

Brief Description of the Figures

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Figure 1 is an illustration of the chemical structures of β -L-2',3'-dideoxycytidine (β -L-FddC), β -D-2',3'-dideoxycytidine (β -D-ddC), β -L-2',3'-dideoxy-5-fluorocytidine (β -L-ddC), (-)- β -L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ((-)- β -L-FTC), (+)- β -D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-dioxolane ((+)- β -D-FDOC),
15 and β -L-2-amino-6-(R⁴)-9-[(4-hydroxymethyl)-tetrahydrofuran-1-yl]purine.

Figure 2 is an illustration of the numbering scheme used in the chemical nomenclature for nucleosides in this text.

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Detailed Description of the Invention

As used herein, the term "enantiomerically pure" refers to a nucleoside composition that includes at least approximately 95%, and preferably approximately 97%, 98%, 99%, or 100% of a single enantiomer of that
25 nucleoside.

The term alkyl, as used herein, unless otherwise specified, refers to a saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbon of C₁ to C₁₀, and specifically includes methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *t*-butyl, pentyl, cyclopentyl, isopentyl,

neopentyl, hexyl, isohexyl, cyclohexyl, cyclohexylmethyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. The alkyl group can be optionally substituted with one or more moieties selected from the group consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, 5 nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., "Protective Groups in Organic Synthesis," John Wiley and Sons, Second Edition, 1991. The term lower alkyl, as used herein, and unless otherwise 10 specified, refers to a C₁ to C₄ ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, or t-butyl group.

As used herein, the term acyl specifically includes but is not limited to acetyl, propionyl, butyryl, pentanoyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, benzoyl, acetyl, pivaloyl, mesylate, 15 propionyl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic.

The term aryl, as used herein, and unless otherwise specified, refers to phenyl, biphenyl, or naphthyl, and preferably phenyl. The aryl group can be optionally substituted with one or more moieties selected from the group 20 consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., "Protective Groups in Organic Synthesis," John Wiley and Sons, Second Edition, 25 1991.

The term purine or pyrimidine base includes, but is not limited to, adenine, N⁶-alkylpurines, N⁶-acylpurines (wherein acyl is C(O)(alkyl, aryl, alkylaryl, or arylalkyl), N⁶-benzylpurine, N⁶-halopurine, N⁶-vinylpurine, N⁶-acetylenic purine, N⁶-acyl purine, N⁶-hydroxyalkyl purine, N⁶-thioalkyl

purine, N²-alkylpurines, N²-alkyl-6-thiopurines, thymine, cytosine, 6-azapyrimidine, 2- and/or 4-mercaptopyrimidine, uracil, C⁵-alkylpyrimidines, C⁵-benzylpyrimidines, C⁵-halopyrimidines, C⁵-vinylpyrimidine, C⁵-acetylenic pyrimidine, C⁵-acyl pyrimidine, C⁵-hydroxyalkyl purine, C⁵-amidopyrimidine, C⁵-cyanopyrimidine, C⁵-nitropyrimidine, C⁵-aminopyrimidine, N²-alkylpurines, N²-alkyl-6-thiopurines, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, pyrazolopyrimidinyl. Functional oxygen and nitrogen groups on the base can be protected as necessary or desired. Suitable protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, *t*-butyldimethylsilyl, and *t*-butyldiphenylsilyl, trityl, alkyl groups, acyl groups such as acetyl and propionyl, methylsulfonyl, and *p*-toluylsulfonyl.

As used herein, the term natural amino acid includes but is not limited to alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaoyl, lysinyl, argininyl, and histidinyl.

The invention as disclosed herein is a method and composition for the treatment of HBV infection and other viruses replicating in a like manner, in humans or other host animals, that includes administering an effective amount of one or more of the above-identified compounds, or a physiologically acceptable derivative, or a physiologically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier. The compounds of this invention either possess anti-HBV activity, or are metabolized to a compound or compounds that exhibit anti-HBV activity.

I. Structure and Preparation of Active Nucleosides

Stereochemistry

The compounds used in the methods disclosed herein are enantiomers of 2',3'-dideoxycytidine, 2',3'-dideoxy-5-(halo or methyl)cytidine, 2-
5 hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-dioxolane, or 2-amino-6-(OH, Cl, NH₂, or H)-9-[(4-hydroxymethyl)-tetrahydrofuran-1-yl]purine.

Since the 1' and 4' carbons of the sugar or dioxolanyl moiety (referred to below generically as the sugar moiety) of the nucleosides are chiral, their nonhydrogen substituents (CH₂OR and the pyrimidine or purine base,
10 respectively) can be either cis (on the same side) or trans (on opposite sides) with respect to the sugar ring system. The four optical isomers therefore are represented by the following configurations (when orienting the sugar moiety in a horizontal plane such that the "primary" oxygen (that between the C1' and C4'-atoms; see Figure 2) is in back): cis (with both
15 groups "up", which corresponds to the configuration of naturally occurring nucleosides), cis (with both groups "down", which is a nonnaturally occurring configuration), trans (with the C2 substituent "up" and the C5 substituent "down"), and trans (with the C2 substituent "down" and the C5 substituent "up"). As indicated schematically in Figure 1, the "D-
20 nucleosides" are cis nucleosides in a natural configuration and the "L-nucleosides" are cis nucleosides in the nonnaturally occurring configuration.

The nucleosides useful in the disclosed method to treat HBV infection are β -L-enantiomers, with the exception of FDOC, which is used in its β -
25 D-enantiomeric form, because it has been discovered that the β -D-enantiomer of FDOC is surprisingly less toxic than the β -L-enantiomer of FDOC.

Prodrug Formulations

The nucleosides disclosed herein can be administered as any derivative that upon administration to the recipient, is capable of providing directly or indirectly, the parent active compound, or that exhibits activity in itself. In
5 one embodiment, the hydrogen of the 5'-OH group is replaced by a C₁-C₂₀ alkyl, including C₁ to C₃ alkyl; acyl in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic C₁-C₂₀ alkyl including C₁ to C₃ alkyl, phenyl, or benzyl; a naturally occurring or nonnaturally occurring amino acid; alkoxyalkyl including methoxymethyl;
10 aralkyl including benzyl; aryloxyalkyl such as phenoxymethyl; aryl including phenyl optionally substituted with halogen, C₁ to C₄ alkyl or C₁ to C₄ alkoxy; a dicarboxylic acid such as succinic acid; sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl; or a mono, di or triphosphate ester.

15 One or both hydrogens of the amino groups on the purine or pyrimidine base can be replaced by a C₁-C₂₀ alkyl, including C₁ to C₃ alkyl; acyl in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic C₁-C₂₀ alkyl, including C₁ to C₃ alkyl, phenyl, or benzyl; alkoxyalkyl including methoxymethyl; aralkyl including
20 benzyl; aryloxyalkyl such as phenoxymethyl; aryl including phenyl optionally substituted with halogen, C₁ to C₄ alkyl or C₁ to C₄ alkoxy.

The active nucleoside can also be provided as a 5'-ether lipid, as disclosed in the following references, which are incorporated by reference herein: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E.J., D.
25 L.W., and C. Piantadosi. 1990. Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation. AIDS Res Hum Retroviruses. 6:491-501; Piantadosi, C., J. Marasco C.J., S.L. Morris-Natschke, K.L. Meyer, F. Gumus, J.R. Surles, K.S. Ishaq, L.S. Kucera, N. Iyer, C.A. Wallen, S. Piantadosi, and

- E.J. Modest. 1991. Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity. *J Med Chem.* **34**:1408.1414; Hostetler, K.Y., D.D. Richman, D.A. Carson, L.M. Stuhmiller, G.M. T. van Wijk, and H. van den Bosch. 1992. Greatly enhanced inhibition of
5 human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3'-deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 3,-deoxythymidine. *Antimicrob Agents Chemother.* **36**:2025.2029; Hostetler, K.Y., L.M. Stuhmiller, H.B. Lenting, H. van den Bosch, and D.D. Richman, 1990. Synthesis and antiretroviral activity
10 of phospholipid analogs of azidothymidine and other antiviral nucleosides. *J. Biol Chem.* **265**:6112.7.

Nucleotide Prodrugs

- Any of the nucleosides described herein, or any other nucleoside that
15 has anti-hepatitis B activity, can be administered as a nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of nucleotide prodrug ligands are known. A nucleotide prodrug, as described herein, refers to a nucleoside that has a phosphate derivative on the 5'-position that is more stable in vivo
20 than the parent phosphate, and which does not materially adversely affect the anti-hepatitis B activity of the nucleoside. Phosphonates are included as phosphate derivatives. In general, alkylation, acylation or other lipophilic modification of the mono, di or triphosphoate of the nucleoside will increase the stability of the nucleotide. Examples of substituent groups that
25 can replace one or more hydrogens on the the phosphate moiety are alkyl, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bischofberger, *Antiviral Research*, **27** (1995) 1-17. Any of these can be used in combination with

the disclosed nucleosides to achieve a desired effect. Numerous examples of nucleotide prodrugs are described in the following references.

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Preparation of the Active Compounds

- The nucleosides used in the disclosed method to treat HBV infections in a host organism can be prepared according to published methods. 8-L-Nucleosides can be prepared from methods disclosed in, or standard modifications of methods disclosed in, for example, the following publications: Jeong, et al., *J. of Med. Chem.*, 36, 182-195, 1993; European Patent Application Publication No. 0 285 884; Genu-Dellac, C., Gosselin, A.-M., Aubertin, G., Obert, A., Kim, and J.-L. Imbach, 3-Substituted thymine α -L-nucleoside derivatives as potential antiviral agents; synthesis and biological evaluation, *Antiviral Chem. Chemother.* 2:83-92 (1991); Johansson, K. N. G., B. G. Lindborg, and R. Noreen, European Patent Application 352 248; Mansuri, M. M., V. Farina, J. E. Starrett, D. A. Benigni, V. Brankovan, and J. C. Martin, Preparation of the geometric isomers of DDC, DDA, D4C and D4T as potential anti-HIV agents,

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- Bioorg. Med. Chem. Lett. 1:65-68 (1991); Fujimori, S., N. Iwanami, Y. Hashimoto, and K. Shudo, A convenient and stereoselective synthesis of 2'-deoxy- β -L-ribonucleosides, Nucleosides & Nucleotides 11:341-349 (1992); Genu-Dellac, C., G. Gosselin, A.-M. Aubertin, G. Obert, A. Kim, and J.-L. Imbach, 3-Substituted thymine α -L-nucleoside derivatives as potential antiviral agents; synthesis and biological evaluation, Antiviral Chem. Chemother. 2:83-92 (1991); Holy, A., Synthesis of 2'-deoxy-L-uridine, Tetrahedron Lett. 2:189-192 (1992); Holy, A., Nucleic acid components and their analogs. CLIII. Preparation of 2'-deoxy-L-ribonucleosides of the pyrimidine series. Collect Czech Chem Commun. 37:4072-4087 (1992); Holy, A, 2'-deoxy-L-uridine: Total synthesis of a uracil 2'-deoxynucleoside from a sugar 2-aminooxazoline through a 2,2'-anhydronucleoside intermediate. In: Townsend LB, Tipson RS, ed. Nucleic Acid Chem. New York: Wiley, 1992: 347-353. vol 1) (1992); Okabe, M., R.-C. Sun, S. Tan, L. Todaro, and D. L. Coffen, Synthesis of the dideoxynucleosides ddC and CNT from glutamic acid, ribonolactone, and pyrimidine bases. J Org Chem. 53:4780-4786 (1988); Robins, M. J., T. A. Khwja, and R. K. Robins. Purine nucleosides. XXIX. Synthesis of 2'-deoxy-L-adenosine and 2'-deoxy-L-guanosine and their alpha anomers. J Org Chem. 35:363-639 (1992); Genu-Dellac, C., Gosselin G., Aubertin A-M, Obert G., Kim A., and Imbach J-L, 3'-Substituted thymine α -L-nucleoside derivatives as potential antiviral agents; synthesis and biological evaluation. Antiviral Chem. Chemother. 2(2):83-92 (1991); Genu-Dellac, C., Gosselin G., Imbach J-L; Synthesis of new 2'-deoxy-3'-substituted- α -L-threo-pentofuranonucleosides of thymine as a potential antiviral agents. Tet Lett 32(1):79-82 (1991); Genu-Dellac, C., Gosselin G., Imbach J-L. Preparation of new acylated derivatives of L-arabino-furanose and 2-deoxy-l-erythro-pentofuranose as precursors for the synthesis of l-pentofuranosyl nucleosides. 216:240-255 (1991); and Genu-

Dellac, C., Gosselin G., Puech F, et al. Systematic synthesis and antiviral evaluation of α -L-arabinofuranosyl and 2'-deoxy- α -L-erythro-pentofuranosyl nucleosides of the five naturally occurring nuclei acid bases. 10(b):1345-1376 (1991).

5 2',3'-Dideoxycytidine (DDC) is a known compound. The D-enantiomer of DDC is currently being marketed by Hoffman-LaRoche under the name Zalcitabine for use in the treatment of persons infected with HIV. See U.S. Patent Nos. 4,879,277 and 4,900,828.

Enantiomerically pure β -D-dioxolane-nucleosides such as β -D-FDOC
10 can be prepared as disclosed in detail in PCT/US91/09124. The process involves the initial preparation of (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane from 1,6-anhydromannose, a sugar that contains all of the necessary stereochemistry for the enantiomerically pure final product, including the correct diastereomeric configuration about the
15 1 position of the sugar (that becomes the 4'-position in the later formed nucleoside). The (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane is condensed with a desired heterocyclic base in the presence of SnCl_4 , other Lewis acid, or trimethylsilyl triflate in an organic solvent such as dichloroethane, acetonitrile, or methylene chloride, to
20 provide the stereochemically pure dioxolane-nucleoside.

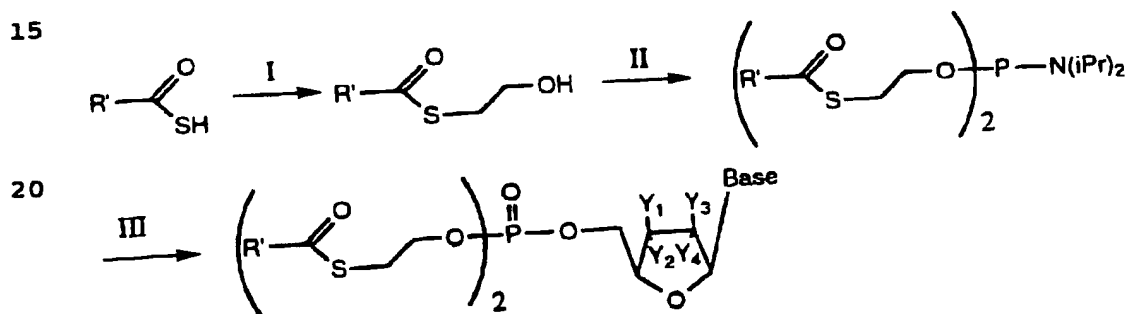
Enzymatic methods for the separation of D and L enantiomers of cis-nucleosides are disclosed in, for example, Nucleosides and Nucleotides, 12(2), 225-236 (1993); European Patent Application Nos. 92304551.2 and 92304552.0 filed by Biochem Pharma, Inc.; and PCT Publication Nos.
25 WO 91/11186, WO 92/14729, and WO 92/14743 filed by Emory University.

Separation of the acylated or alkylated racemic mixture of D and L enantiomers of cis-nucleosides can be accomplished by high performance

liquid chromatography with chiral stationary phases, as disclosed in PCT Publication No. WO 92/14729.

Mono, di, and triphosphate derivative of the active nucleosides can be prepared as described according to published methods. The
 5 monophosphate can be prepared according to the procedure of Imai et al., I. Org. Chem., 34(6), 1547-1550 (June 1969). The diphosphate can be prepared according to the procedure of Davisson et al., I. Org. Chem., 52(9), 1794-1801 (1987). The triphosphate can be prepared according to the procedure of Hoard et al., I. Am. Chem. Soc., 87(8), 1785-1788
 10 (1965).

General Procedures for the Preparation of Bis (S-acyl-2-thioethyl) Phosphoester of β -L-dideoxynucleosides [Bis (SATE) β -L ddx MP]

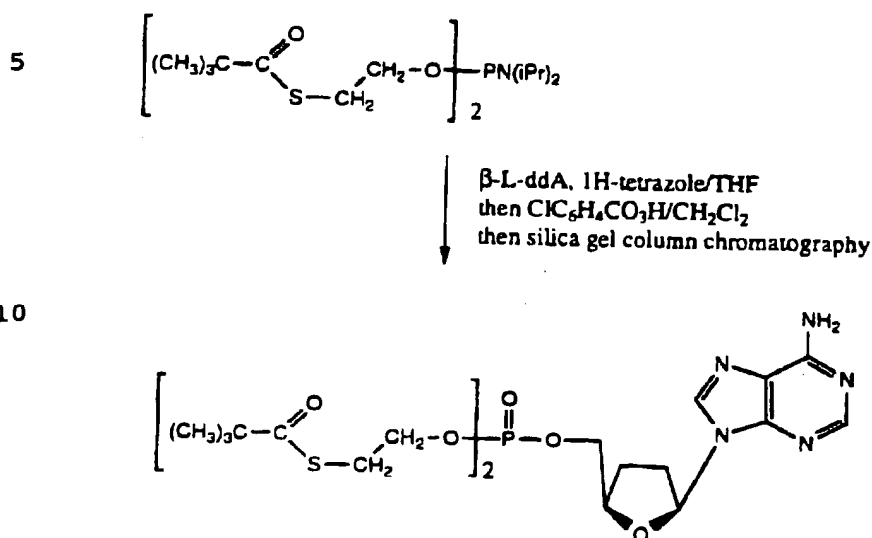


Bis (SATE) β -L-ddxMP

30 Y^1 , Y^2 , Y^3 , and Y^4 are independently H, OH, N_3 , NR^1R^2 , NO_2 , NOR^3 , -O-alkyl,

- O-aryl, halo (including F, Cl, Br, or I), -CN, -C(O)NH₂, SH, -S-alkyl, or -S-aryl, and wherein typically three of Y¹, Y², Y³, and Y⁴ are either H or OH. The -OH substituent, when present, is typically a Y¹ or Y³ group. As illustrated in the structure, Y² and Y⁴ are in the arabino (erythro) configuration, and Y¹ and Y³ are in the threo (ribose) configuration. The base is a purine or pyrimidine. Alternatively, the psuedo-sugar moiety is a 1,3-oxathiolane (as in FTC and BCH-189 or 3TC or is a 1,3-dioxolane derivative). (i) ICH₂CH₂OH, DBU/C₆H₅CH₃; (ii) Cl₂PN(iPr)₂, NEt₃/THF; (iii) β-L-dideoxynucleoside, 1H--tetrazole/THF, then
- 5 configuration, and Y¹ and Y³ are in the threo (ribose) configuration. The base is a purine or pyrimidine. Alternatively, the psuedo-sugar moiety is a 1,3-oxathiolane (as in FTC and BCH-189 or 3TC or is a 1,3-dioxolane derivative). (i) ICH₂CH₂OH, DBU/C₆H₅CH₃; (ii) Cl₂PN(iPr)₂, NEt₃/THF; (iii) β-L-dideoxynucleoside, 1H--tetrazole/THF, then
- 10 ClC₆H₄CO₃H/CH₂Cl₂ 1H-Tetrazole (0.21g, 3.0 mmol) was added to a stirred solution of β-L-dideoxynucleoside (1.0 mmol) and the appropriate phosphoramidite C (1.2 mmol) in tetrahydrofuran (2mL) at room temperature. After 30 minutes, the reaction mixture was cooled to -40°C and a solution of 3-chloroperoxybenzoic acid (0.23 g, 1.3 mmol) in
- 15 dichloromethane (2.5 mL) was added; the mixture was then allowed to warm to room temperature over 1 h. Sodium sulfite (10% solution, 1.3 mL) was added to the mixture to destroy the excess 3-chloroperoxybenzoic acid, after which the organic layer was separated and the aqueous layer washed with dichloromethane (2 x 10 mL). The combined organic layers
- 20 were washed with saturated aqueous sodium hydrogen carbonate (5 mL), then water (3 x 5 mL), dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure. Column chromatography of the residue on silica gel afforded the title Bis(SATE) β-L-ddxmP.

EXAMPLE = β -L-2',3'-Dideoxyadenosin-5'-yl bis (2-pivaloylthioethyl) phosphate [Bis (SATE) β -L-ddAMP].



Bis (SATE) β -L-ddAMP

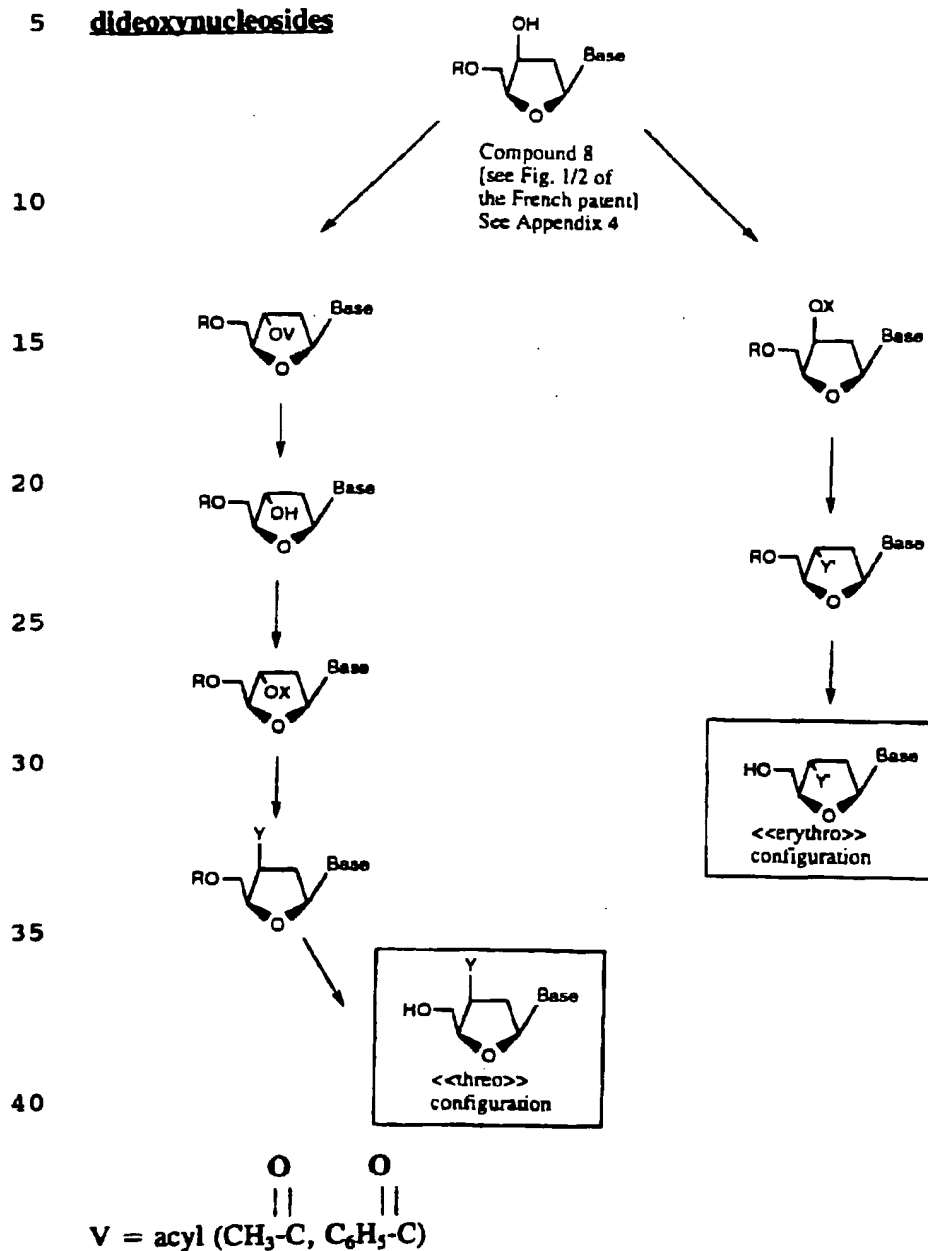
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Following the above general procedure, pure Bis(SATE) β -L-ddAMP was obtained as a colorless oil in 72% yield after silica gel column chromatography [eluent: stepwise gradient of methanol (0-3%) in dichloromethane]; ^1NMR ($\text{DMSO}-d_6$) δ ppm: 8.26 and 8.13 (2s, 2H each, H-2 and H-8), 7.20 (br s, 2H, NH_2), 6.24 (t, 1H, H-1'; $J=6.0$ Hz), 4.35 - 4.25 (m, 1H, H-4'), 4.25-4.00 (m, 2H, H-5', 5''), 3.96 (m, 4H, 2 $\text{SCH}_2\text{CH}_2\text{O}$), 3.04 (t, 4H, 2 $\text{SCH}_2\text{CH}_2\text{O}$; $J = 6.3$ Hz), 2.5 - 2.4 (m, 2H, H-2', 2''), 2.2-2.0 (m, 2H, H-3', 3''), 1.15 [s, 18H, 2 $(\text{CH}_3)_3\text{C}$]; ^{31}P -NMR ($\text{DMSO}-d_6$) δ ppm = -0.76 (s); UV (EtOH), $\lambda_{\text{max}} = 259$ nm (ϵ 15400);

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mass spectrum (performed in: glycerol, thioglycerol, 1:1, v/v), FAB > O
604 (M+H)⁺, 136 (BH₂)⁺.

**General scheme for the stereospecific synthesis of 3'-substituted β-L-
dideoxynucleosides**



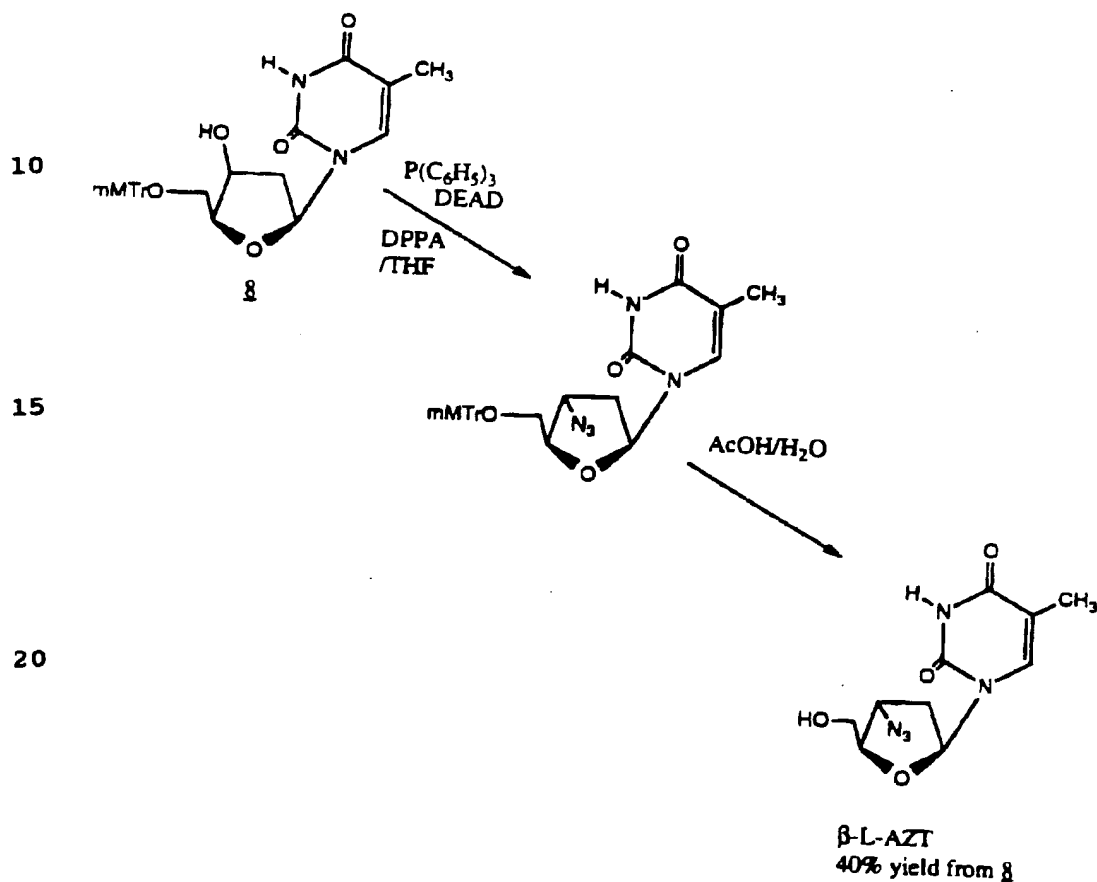
X = Leaving group [CH_3 , SO_2 , CH_3 , C_6H_4 , SO_2 , CF_3 , SO_2]

Y, Y' = F, N_3 , NR_1R_2 [R_1, R_2 = H, alkyl, aryl],

NO_2 , NOR [R = H, alkyl, acyl], O-alkyl, O-aryl, etc.

EXAMPLE = 1-(3-Azido-2,3-dideoxy- β -L-erythro-pentofuranosyl)

5 thymine [β -L-AZT]



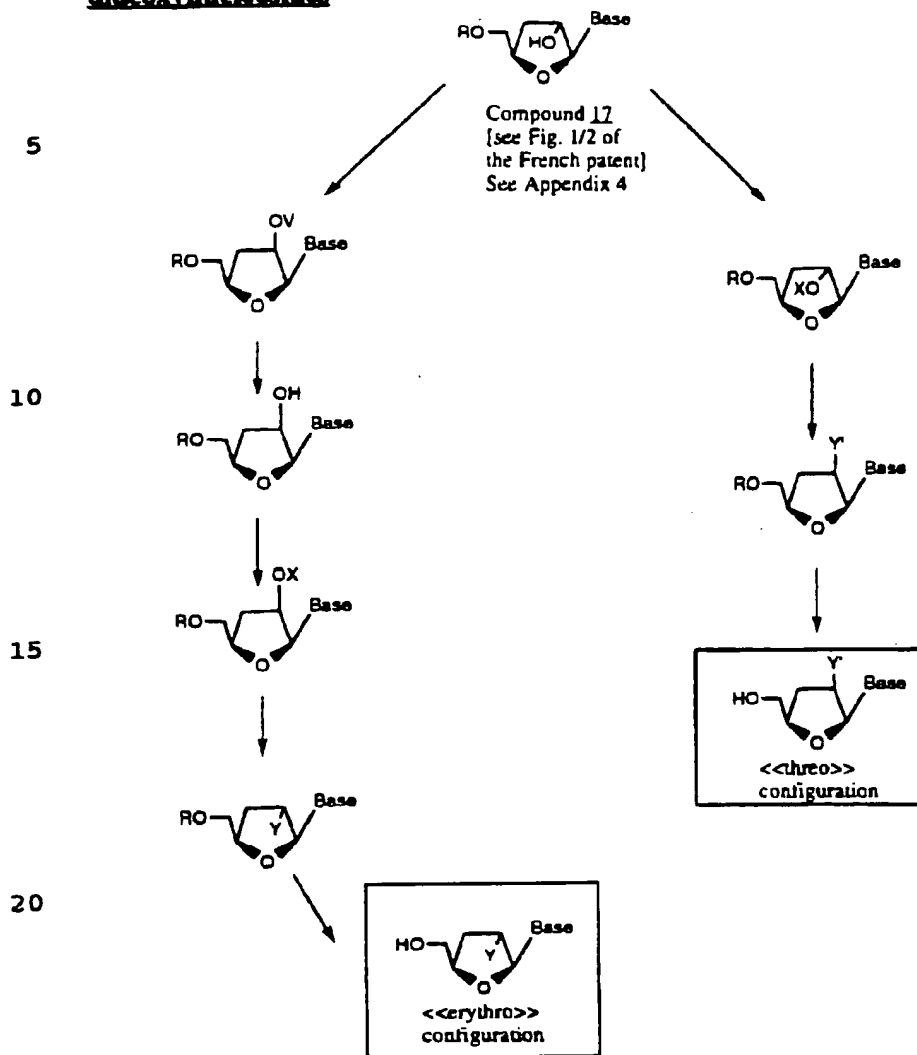
A mixture of diethyl azodicarboxylate (0.46 mL; 2.9 mmol) and diphenyl phosphorazidate (0.62 mL; 2.9 mmol) in THF (2.9 mL) was added dropwise over 30 min. to a solution of 1-(2-deoxy-5-O-monomethoxytrityl- β -L-threo-pentofuranosyl) thymine **8** [0.5 g, 0.97 mmol] and

5 triphenylphosphine (0.76g, 2.9 mmol) in THF 11.6 mL) at 0°C. The mixture was stirred for 3.5h at room temperature, and ethanol was added. After concentration to dryness in vacuo, the residue was dissolved in a mixture of acidic acid (240 mL) and water (60 mL) in order to remove the mMTTr protecting group. The mixture was stirred for 5 hours at room

10 temperature and was diluted with toluene. The separated aqueous phase was concentrated to dryness in vacuo. The residue was purified over a silica gel column eluted with ethyl acetate to afford β -L-AZT (105 mg, 40%, crystallized from ethyl acetate). The physicochemical data of β -L-AZT were in accordance with literature data [J. Wengel, J-Lau, E.B. Ledersen, C.N. Nielsen, J. Org. Chem. **56** (11), 3591-3594 (1991)].

15

General Scheme for the Stereospecific Synthesis of 2'-substituted β -L-dideoxynucleosides



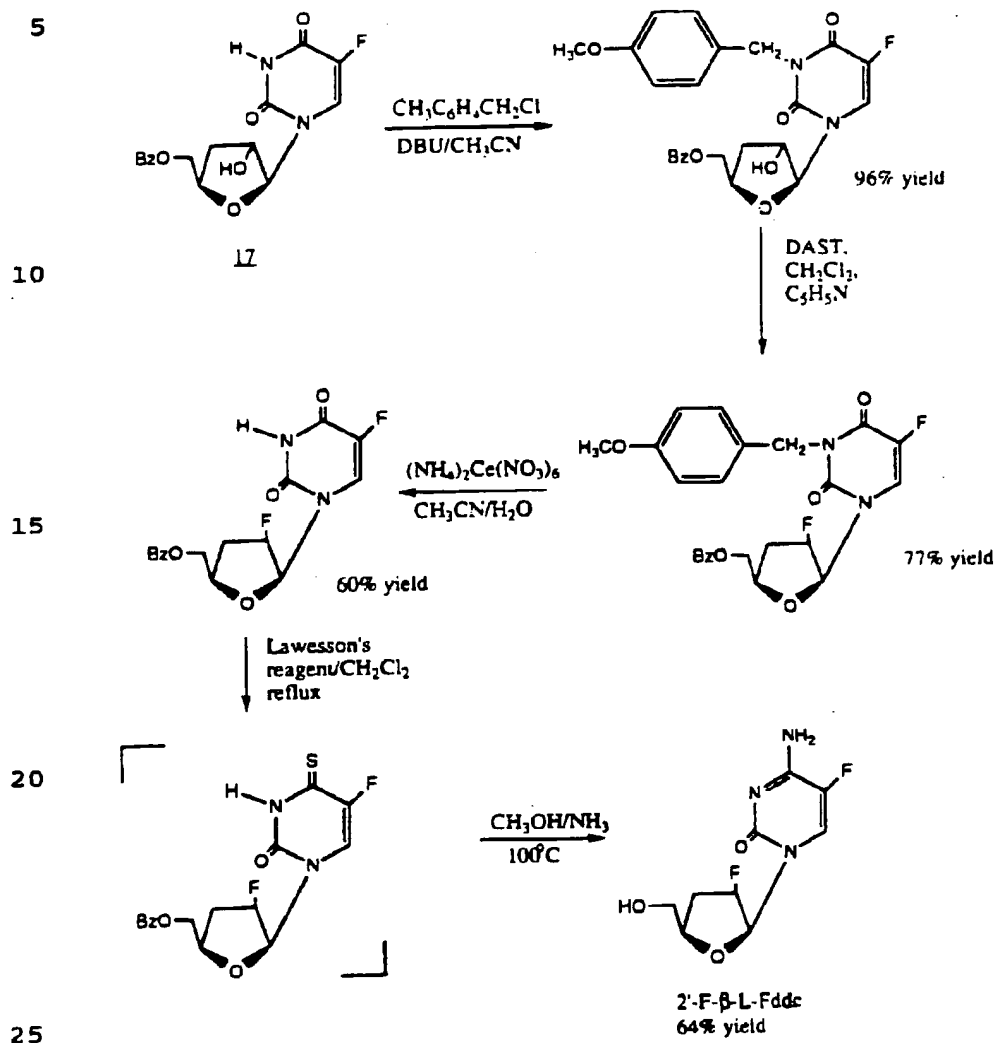
V = acyl [$\text{CH}_3\text{-C}(=\text{O})\text{C}_6\text{H}_5\text{-C}(=\text{O})$]

X = Leaving group [CH_3SO_2 , $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{H}$, CF_3SO_2]

Y, Y' = F, N_3 , NR_1R_2 [R_1, R_2 = H, alkyl, aryl],

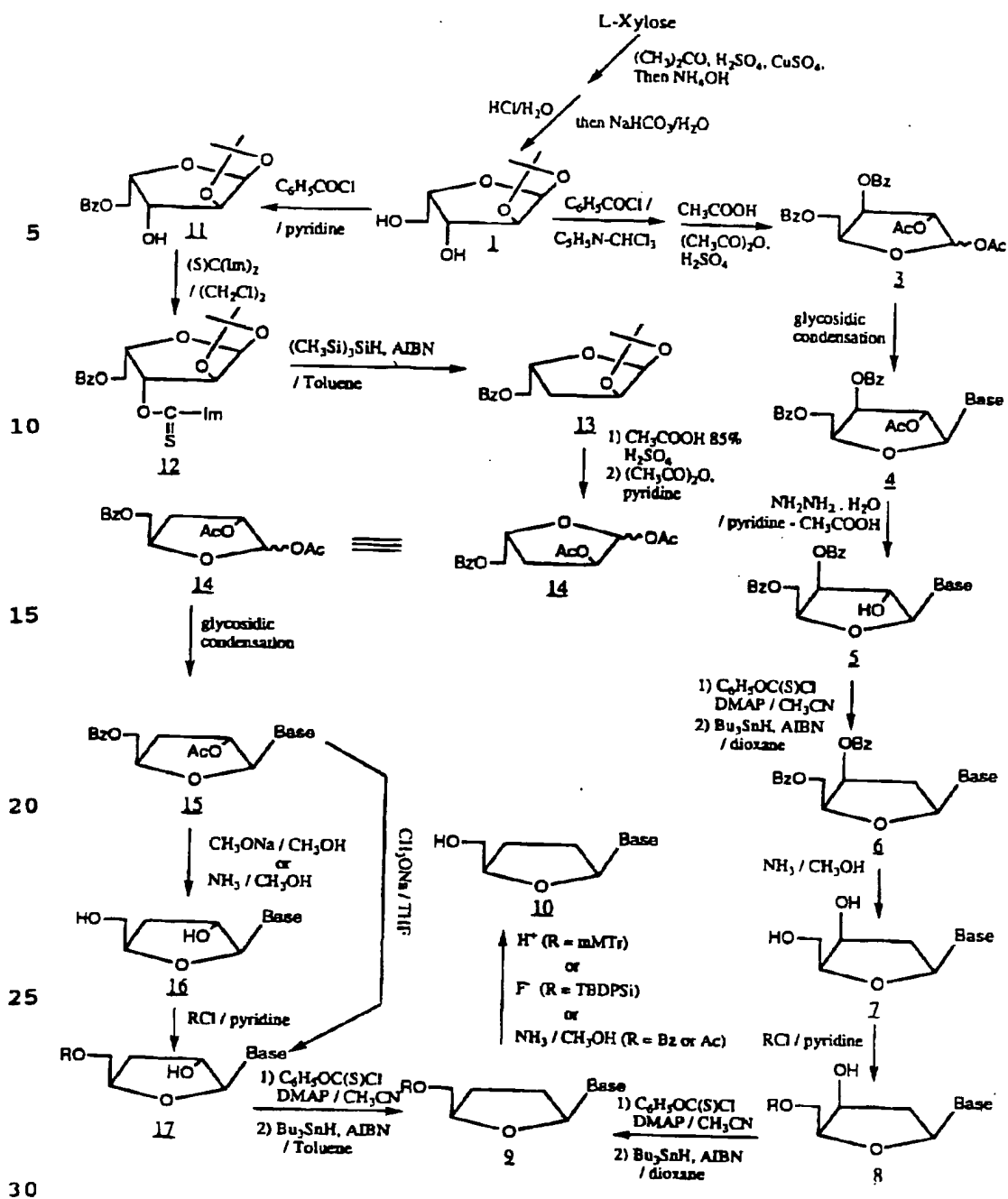
NO_2 , NOR [R = H, alkyl, acyl], O-alkyl, O-aryl, etc.

EXAMPLE = 1-(2-Fluoro-2,3-dideoxy- β -L-threo-pentofuran syl)-5-fluorocytosine [2'-F- β -L β -L-FddC]



Hitherto unknown 2'-F- β -L-FddC was synthesized in five steps from 1-(5-O-benzoyl-3-deoxy- β -L-erythro-pentofuranosyl)-5-fluorouracil 17 with an overall yield of 28%. m.p. $209-210^\circ\text{C}$ (crystallized from absolute

ethanol); UV (Et OH) λ_{\max} 276 nm (ϵ , 9000), λ_{\min} 226 nm (ϵ , 4000); ^{19}F -
NMR (DMSO- d_6) δ ppm : -179.7 (m, $\text{F}_{2'}$) , -167.2 (dd, F_5 ; $J_{\text{F},6} = 7.3$ Hz,
 $J_{\text{F},1'} = 1.5$ Hz); ^1H -NMR (DMSO- d_6) δ ppm : 8.30 (d , 1H, H-6; $J_{6,\text{F}} = 7.3$
Hz), 7.8-7.5 (br s, 2H, NH_2), 5.80 (d, 1H, H-1' $J_{1',\text{F}} = 17.4$ Hz), 5.34 (t,
5 1H, OH-5'; $J = 4.8$ Hz), 5.10 (dd, 1H, H-2'; $J_{2',\text{F}} = 51.2$ Hz; $J_{2',3'} =$
3.4 Hz), 4.3 (m, 1H, H-4'), 3.8-3.6 (m, 2H, H-5',5''), 2.2-2.0 (m, 2H,
H-3', H-3''); mass spectra (performed in: glycerol-thioglycerol, 1:1 v/v),
FAB>O:248 ($\text{M}+\text{H}$) $^+$, 130 (BH_2) $^+$; FAB<O:246 ($\text{M}-\text{H}$) $^-$; $[\alpha]_{\text{D}}^{20} = -$
16.5 ($-c$ 0.85, DMSO). Anal. Calc. for $\text{C}_9\text{H}_{11}\text{N}_3\text{O}_3\text{F}_2$: C, 43.73; H,
10 9.49; N, 17.00; F, 15.37 . Found: C, 43.56; H,
4.78; N, 16.75; F, 14.96.



Scheme I: Bases = purines or pyrimidines, eventuellement convenablement protégées; R
 = Benzoyl (Bz) Acetyl (Ac), monomethoxytrityl (mMTr) or tertiarybutyldiphenylsilyl
 (TBDPSi)

II. Anti-HBV Activity of Nucleosides

The ability of the active compounds to inhibit HBV can be measured by various experimental techniques. The assay used herein to evaluate the ability of the disclosed compounds to inhibit the replication of HBV is described in detail in Korba and Gerin, *Antiviral Res.* 19: 55-70 (1992). For purposes of illustration only, and without limiting the invention, the results of the evaluation of toxicity and anti-HBV activity are provided below for β -L-2',3'-dideoxycytidine (β -L-FddC), β -L-2',3'-dideoxy-5-fluorocytidine (β -L-ddC), and (+)- β -D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-dioxolane ((+)- β -D-FDOC). The toxicity and anti-HBV activity of (-)- β -L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ((-)- β -L-FTC) and β -D-2',3'-dideoxycytidine (β -D-ddC) are included as controls. The other compounds disclosed herein can be evaluated similarly.

The samples of β -L-ddC and β -L-5-FddC used in the anti-HBV assays were characterized as follows.

2',3'-Dideoxy- β -L-cytidine (β -L-DDC). m.p. = 220-220°C; UV (EtOH 95) max 273 nm, λ_{\min} 252 nm; NMR- ^1H (DMSO- d_6) δ ppm = 7.89 (d, 1H, H-6; J = 7.4 Hz). 7.15-6.95 (d large, 2H, NH_2), 5.91 (dd, 1H, H-1'; J = 3.0 et 6.5 Hz), 5.66 (d, 1H, H-5; J = 7.4 Hz), 4.99 [t, 1H, OH-5'; J = 5.2 Hz]. 4.05-3.95 (m, 1H, H-4'), 3.60-3.70 (m, 1H, H-5'; after D_2O exchange: dd, 3.64 ppm, J = 3.6 et 12.0 Hz). 3.60-3.50 (m, 1H, H-5"; after D_2O exchange: dd, 3.50 ppm, J = 4.1 et 12.0 Hz), 2.30-2.15 (m, 1H, H-2'), 1.9-1.65 (m, 3H, H-2", 3' et 3"); $[\alpha]_D^{20}$ -103.6 (c 0.8 MeOH); mass spectrum (performed in: glycerol-thioglycerol, 50 : 50. v/v); FAB > 0 423 $[\text{2M} + \text{H}]^+$, 304 $[\text{M} + \text{glycerol} + \text{H}]^+$. 212 $[\text{M} + \text{H}]^+$, 112 $[\text{BH}_2]^+$, 101 $[\text{s}]^+$; FAB < 0 210 $[\text{M} - \text{H}]^-$. *Anal. Calc. for*

$C_9H_{13}N_3O_3$ ($M = 211.21$); C 51.18; H 6.20; N 19.89 found; C 51.34; H 6.25; N 20.12.

2',3'-Dideoxy-8-L-5-fluorocytidine (8-L-5-FDDC). m.p. = 158-160°C; UV (EtOH 95) λ_{max} 281 nm (ϵ , 8100) et 237 nm (ϵ , 8500); min
 5 260 nm (ϵ , 5700) et 225 nm (ϵ , 7800); NMR - 1H (DMSO- d_6) δ ppm 8.28 (d, 1H, H-6; J - 7.4 Hz), 7.7-7.4 (d large, 2H, NH_2), 5.83 (dd poorly resolved, 1H, H-1'), 5.16(t, 1H, OH-5'; J = 5.1 Hz), 4.05-3.95 (m, 1H, H-4'), 3.8-3.70 [m, 1H, H 5'; after D2O exchange: dd, 3.71 ppm. J = 2.7 et 12.3 Hz], 3.60-3.50 [m, 1H, H-5"; after D₂O exchange: dd, 3.52 ppm;
 10 J = 3.3 et 12.3 Hz], 2.35-2.15 (m, 1H, H-2'). 1.95-1.75 (m, 3H, H-2", 3' et 3"): $[\alpha]_D^{20}$ -80.0 (-c 1.0, DMSO); Mass spectrum [performed in: 3-nitrobenzyl alcohol] FAB > 0 230 $[M+H]^+$ et 101 $[s]^+$; FAB < 0 228 $[M-II]^+$. Anal. Calculated for $C_9H_{12}N_3FO_3$ ($M = 229.21$); C 47.16; H 5.28; N 18.33, F 8.29, Found. C 46.90; H 5.28; N 18.07; F 8.17.

15 The antiviral evaluations were performed on two separate passages of cells, two cultures per passage (4 cultures total). All wells, in all plates, were seeded at the same density and at the same time.

Due to the inherent variations in the levels of both intracellular and extracellular HBV DNA, only depressions greater than 3.0-fold (for HBV
 20 virion DNA) or 2.5-fold (for HBV DNA replication intermediates) from the average levels for these HBV DNA forms in untreated cells are generally considered to be statistically significant [$P < 0.05$] (Korba and Gerin, Antiviral Res. 19: 55-70, 1992). The levels of integrated HBV DNA in each cellular DNA preparation (which remain constant on a per
 25 cell basis in these experiments) were used to calculate the levels of intracellular HBV DNA forms, thereby eliminating technical variations inherent in the blot hybridization assays.

Typical values for extracellular HBV virion DNA in untreated cells range from 50 to 150 pg/ml culture medium (average of approximately 76

pg/ml). Intracellular HBV DNA replication intermediates in untreated cells range from 50 to 100 pg/ug cell DNA (average approximately 74 pg/ug cell DNA). In general, depressions in the levels of intracellular HBV DNA due to treatment with antiviral compounds are less pronounced,
5 and occur more slowly, than depressions in the levels of HBV virion DNA.

For reference, the manner in which the hybridization analyses were performed for these experiments results in an equivalence of approximately 1.0 pg intracellular HBV DNA/ug cellular DNA to 2-3 genomic copies per cell and 1.0 pg of extracellular HBV DNA/ml culture medium to 3×10^5
10 viral particles/ml.

Toxicity analyses were performed in order to assess whether any observed antiviral effects were due to a general effect on cell viability. The method used was based on the uptake of neutral red dye, a standard and widely used assay for cell viability in a variety of virus-host systems,
15 including HSV (herpes simplex virus) and HIV.

The test compounds were used in the form of 40 mM stock solutions in DMSO (frozen on dry ice). Daily aliquots of the test samples were made and frozen at -20°C so that each individual aliquot would be subjected to a single freeze-thaw cycle. The daily test aliquots were thawed, suspended
20 into culture medium at room temperature and immediately added to the cell cultures. The compounds were tested at 0.01 to 10 μM for antiviral activity. The compounds were tested for toxicity at concentrations from 1 to 300 μM . The results are provided in Table 1.

Table 1

**EFFECT OF D-DDC, L-DDC, L-FDDC, FDOC and (-)-FTC AGAINST HEPATITIS B VIRUS
IN TRANSFECTED HEPG-2 (2.2.15) CELLS**

Compound	HBV viron ^a		HBV RI ^b		Cytotoxicity IC ₅₀ _ SD	Selectivity Index IC ₅₀ / EC ₉₀ Viron RI
	EC ₅₀ _ SD	EC ₉₀ _ SD	EC ₅₀ _ SD	EC ₉₀ _ SD		
β-D-DDC	1.3 _ 0.2 ^c	2.1 _ 0.3	8.1 _ 1.7	12.0 _ 2.4	219 _ 28 ^c	104
	1.5 _ 0.7	9.4 _ 2.5	3.2 _ 0.6	11.0 _ 2.0	216 _ 22	23
β-L-DDC	0.033 _ 0.003	1.1 _ 0.2	0.107 _ 0.012	1.8 _ 0.2	493 _ 64	448
β-L-FDDC	0.12 _ 0.01	0.30 _ 0.03	2.8 _ 0.4	4.8 _ 0.6	438 _ 57	1,460
(+)-β-D-FDOC	0.020 _ 0.003	0.195 _ 0.027	0.062 _ 0.012	0.23 _ 0.02	251 _ 23	1,091
(-)-β-L-FTC	0.017 _ 0.005	0.15 _ 0.02	0.049 _ 0.008	0.18 _ 0.03	292 _ 13	1,947
						1,622

^a Extracellular DNA^b Replicative intermediates (Intracellular DNA)^c μM

Example 2 Toxicity Of Compounds

The ability of the active compounds to inhibit the growth of virus in 2.2.15 cell cultures (HepG2 cells transformed with hepatitis virion) was evaluated. As illustrated in Table 1, no significant toxicity (greater than 50% depression of the dye uptake levels observed in untreated cells) was observed for any of the test compounds at the concentrations 100 μ M. The compounds were moderately toxic at 300 μ M, however, all three compounds exhibited less toxicity at this concentration than β -D-ddC. It appears that the IC_{50} of β -L-ddC and β -L-FddC is approximately twice that of β -D-ddC.

Toxicity analyses were performed in 96-well flat bottomed tissue culture plates. Cells for the toxicity analyses were cultured and treated with test compounds with the same schedule as used for the antiviral evaluations. Each compound was tested at 4 concentrations, each in triplicate cultures. Uptake of neutral red dye was used to determine the relative level of toxicity. The absorbance of internalized dye at 510 nm (A_{510}) was used for the quantitative analysis. Values are presented as a percentage of the average A_{510} values (\pm standard deviations) in 9 separate cultures of untreated cells maintained on the same 96-well plate as the test compounds. The percentage of dye uptake in the 9 control cultures on plate 40 was 100 ± 3 . At 150-190 μ M β -D-ddC, a 2-fold reduction in dye uptake (versus the levels observed in untreated cultures) is typically observed in these assays (Korba and Gerin, Antiviral Res. 19: 55-70, 1992).

Example 3 Anti-Hepatitis B Virus Activity

The positive treatment control, β -D-2',3'-dideoxycytosine [β -D-ddC], induced significant depressions of HBV DNA replication at the concentration used. Previous studies have indicated that at 9-12 μ M of β -D-ddC, a 90% depression of HBV RI (relative to average levels in untreated cells) is typically observed in this assay system (Korba and Gerin, Antiviral Res. 19: 55-70, 1992). This is consistent with the data presented in Table 1.

The data presented in Table 1 indicates that all three test compounds ((β -L-FddC), (β -L-ddC), and β -D-FDOC)), were potent inhibitors of HBV replication, causing depression of HBV virion DNA and HBV RI to a degree comparable to, or greater than, that observed following treatment with β -D-ddC.

Example 4

The effect of selected β -L-derivatives against Hepatitis B virus replication in transfected Hep G-2 cells is described in Table 4.

Table 1: Effect of L-derivatives against Hepatitis B virus replication in transfected Hep G-2 (2.2.15) cells.

Compound	HBV Virion ^a EC ₅₀	HBV RI ^b EC ₅₀	Cytotoxicity IC ₅₀	Selectivity Index	
				Virion	IC ₅₀ /EC ₅₀ RI
β-L-ddA	5.0 ^c	5.0	250	50	50
Bis (Sate) β-L-ddAMP	0.45	0.35	200	445	571
β-L-AZT	> 10	> 10	1000	NA	NA
Bis (Sate) β-L-AZTMP	7.5	8	200	27	25
2'-F-β-L-5FDDC	1.7	5.0	210	124	42

^a Extracellular DNA^b Replicative intermediates (Intracellular DNA)^c μM

Example 5

The Comparative inhibitory effect of selected triphosphates on woodchuck hepatitis virus DNA polymerase is set out in Table 5.

- 5 **Table 2: Comparative inhibitory activities of L-nucleoside triphosphates on woodchuck hepatitis virus DNA polymerase and human DNA polymerase α and β .**

Inhibitor	WHB DNA Pol IC ₅₀ (μ M)	DNA Pol α Ki (μ M)	DNA Pol β Ki (μ M)
β -L-AZTPP	0.2	> 100	> 100
β -L-ddATP	2.1	> 100	> 100
3-TC-TP	1.0	> 100	> 100
β -L-5FDDCTP	2.0	> 100	> 100

10 **III. Preparation of Pharmaceutical Compositions**

- The compounds disclosed herein and their pharmaceutically acceptable salts, prodrugs, and derivatives, are useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused
- 15 by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been exposed to HBV.

- 20 Humans suffering from any of these conditions can be treated by administering to the patient an effective HBV-treatment amount of one or a mixture of the active compounds described herein or a pharmaceutically acceptable derivative or salt thereof, optionally in a pharmaceutically acceptable carrier or diluent. The active materials can be administered by

any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a
5 therapeutically effective amount without causing serious toxic effects in the patient treated.

A preferred dose of the active compound for all of the above-mentioned conditions will be in the range from about 1 to 60 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more generally 0.1 to
10 about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent nucleoside to be delivered. If the derivative exhibits activity in itself, the effective dosage can be
15 estimated as above using the weight of the derivative, or by other means known to those skilled in the art. In one embodiment, the active compound is administered as described in the product insert or Physician's Desk Reference for 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), or 2',3'-dideoxy-2',3'-
didehydrothymidine (D4T) for HIV indication.

20 The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. A oral dosage of 50-1000 mg is usually convenient.

Ideally the active ingredient should be administered to achieve peak
25 plasma concentrations of the active compound of from about 0.2 to 70 μ M, preferably about 1.0 to 10 μ M. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The active compound can be provided in the form of pharmaceutically acceptable salts. As used herein, the term pharmaceutically acceptable salts or complexes refers to salts or complexes of the nucleosides that retain the desired biological activity of the parent compound and exhibit minimal, if any, undesired toxicological effects. Nonlimiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, and polygalacturonic acid; (b) base addition salts formed with cations such as sodium, potassium, zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with an organic cation formed from N,N-dibenzylethylene-diamine, ammonium, or ethylenediamine; or (c) combinations of (a) and (b); e.g., a zinc tannate salt or the like.

Modifications of the active compound, specifically at the N⁶ or N⁴ and 5'-O positions, can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species.

The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering

or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil.

In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

The active compound or pharmaceutically acceptable salt or derivative thereof can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the

active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The active compound, or pharmaceutically acceptable derivative or salt thereof can also be mixed with other active materials that do not impair the
5 desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals, including anti-HBV, anti-cytomegalovirus, or anti-HIV agents.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components:
10 a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or
15 phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological
20 saline or phosphate buffered saline (PBS). In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl
25 acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in

5 U.S. Patent No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearyl phosphatidyl ethanolamine, stearyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind

10 a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal

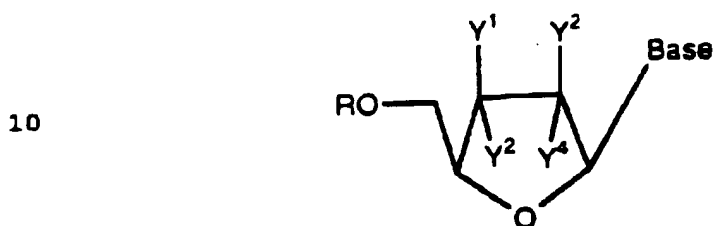
15 suspension.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications

20 be included within the scope of the appended claims.

We claim:

1. A method for the treatment of HBV infection that includes administering a hepatitis B treatment amount of a nucleotide of the
5 formula:



- 15 wherein B is a purine or pyrimidine base;

Y^1 , Y^2 , Y^3 , and Y^4 are independently H, OH, N_3 , NR^1R^2 , NO_2 , NOR^3 ,

- O-alkyl, -O-aryl, halo, -CN, -C(O)NH₂, SH, -S-alkyl, or -S-aryl, wherein typically three of Y^1 , Y^2 , Y^3 , and Y^4 are either H or OH, R is a stabilized
20 phosphate derivative.

2. A method for the treatment of a patient infected with hepatitis B that includes administering a hepatitis B treatment amount of a stabilized nucleotide prodrug of a nucleoside that exhibits antihepatitis B activity.

3. The method of claim 2, wherein the stabilized nucleotide is a
25 SATE derivative.

4. The method of claim 2, wherein the nucleoside is selected from the group consisting of 3TC and FTC.

5. The method of claim 2, wherein the nucleoside is selected from the group consisting of AZT, DDI, D4T, and DDC.

6. The method of claim 2, wherein the nucleoside is selected from the group consisting of dideoxycytidine (8-L-FddC), 8-D-2',3'-dideoxycytidine (8-D-ddC), 8-L-2',3'-dideoxy-5-fluorocytidine (8-L-ddC), (-)-8-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ((-)-8-L-FTC), (+)-8-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-dioxolane ((+)-8-D-FDOC), and 8-L-2-amino-6-(R⁴)-9-[(4-hydroxymethyl)-tetrahydrofuran-1-yl]purine.

10 7. A pharmaceutical composition comprising an effective amount to treat a patient infected with hepatitis B of the nucleotide of claim 1.

8. A pharmaceutical composition comprising an effective amount to treat a patient infected with hepatitis B of the nucleotide of claim 2.

15 9. A pharmaceutical composition comprising an effective amount to treat a patient infected with hepatitis B of the nucleotide of claim 3.

10. A pharmaceutical composition comprising an effective amount to treat a patient infected with hepatitis B of the nucleotide of claim 6.

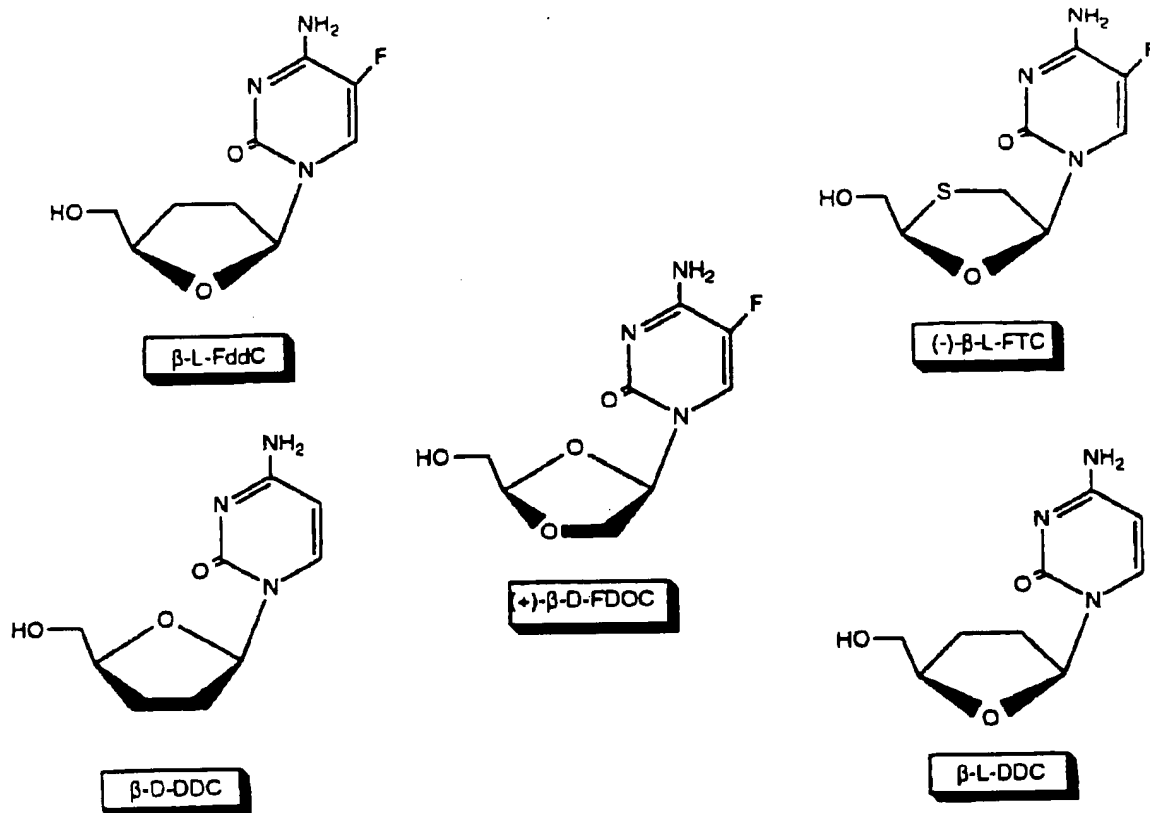


Figure 1

SUBSTITUTE SHEET (RULE 26)

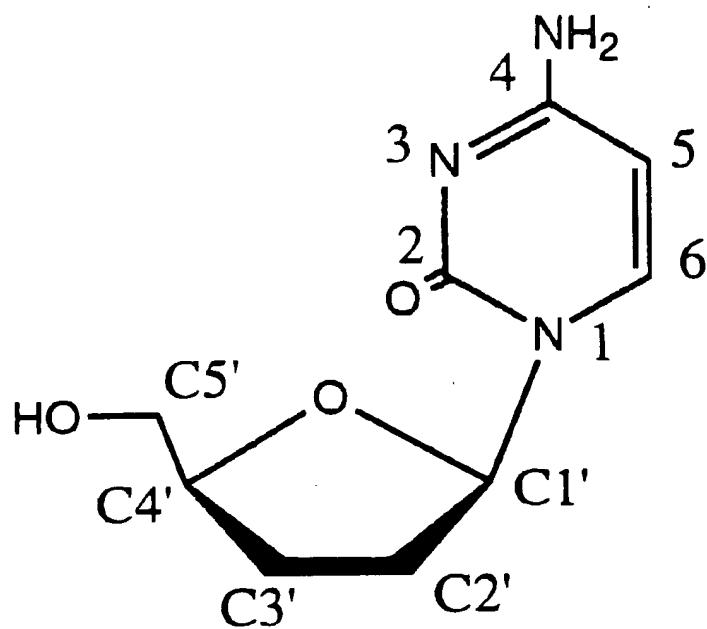


Figure 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/10026

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/70; C07H 19/073, 19/10; 19/173 AND 19/20

US CL : 514/46, 47, 49, 50 AND 51

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/46, 47, 49, 50 AND 51

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Structure search on STN; word search on CAPLUS and APS: keywords: L-nucleosides, HBV, hepatitis B

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ----- A	WO 94/27616 A1 (BIOCHEM PHARMA, INC.) 08 December 1994, page 10, last paragraph to page 11, first structure.	1 - 2, 4-10 <u>3</u>
Y	WO 94/14456 A1 (BIOCHEM PHARMA, INC.) 07 July 1994, see claims 1 - 62.	1 - 10
Y	WO 92/14743 A (EMORY UNIVERSITY) 03 September 1992, see abstract and claims 1 - 58.	1 - 10
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☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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Date of the actual completion of the international search.

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